SOME INFLUENCES OF THE DEVELOPMENT OF HIGHER PLANTS UPON THE MICROORGANISMS IN THE SOIL: VI. MICROSCOPIC EXAMINATION OF THE RHIZOSPHERE[1]

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In previous reports (45–49) it has been shown that microorganisms are much more numerous and active on root surfaces than elsewhere in the soil. The numbers of bacteria increased greatly in response to root development; some increase was also noted in the abundance of filamentous fungi and actinomycetes. As was to be expected, the microorganisms responded differently to different plants and to the various stages of growth of any one plant. In addition to the numerous reports discussed previously, there have been several recent publications leading to the conclusion that microbial activity in soils is greatly favored by plant development. Thom and Humfeld (53) observed also that parasitic attack of roots was accompanied by extensive development of saprophytes. Part of the increase about roots of healthy plants might be ascribed to the fact that the plants modified the reaction of the soil about the roots, acid soils becoming less acid and alkaline soils becoming less alkaline. Reuszer (37) found indications of greater biological activity in pasture soil than in bare soil. McKinley (28) noted greater biological activity under maize, sorghum, wheat, and barley than in fallow soil. Truffaut and Lefouin (56) found that the numbers of bacteria increased during growth of wheat and decreased after the plants were harvested.

The influence of plants is particularly striking in soils of semiarid regions where roots penetrate more deeply than in humid regions. Sabinin and Minina found that some sandy soils of arid regions were practically sterile a short distance from the root systems (43). Roots were largely responsible for the relatively large number of bacteria found in these soils at a depth of 5 m. The results of Krassilnikov, Kriss, and Litvinov indicate that the various groups of soil organisms respond differently to plant development (21, 22). Cellulose-decomposing bacteria seemed to take an active part in the decomposition of the roots, but the predominating bacteria were nonspore-formers.

The present studies were undertaken in order to obtain some concrete pictorial evidence of the relations between small roots and root hairs and the soil organisms. It seemed likely that the contact slide method would be useful for this purpose.[2] Rossi and his associates first reported the new technic

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[2] See preliminary note in *Jour. Bact.* 33: 77 (1937).

by which it was possible to obtain an entirely new conception of the manner in which microorganisms are distributed in the soil (40). Slides or cover slips were pressed against the soil, and the adhering material was stained. Microscopic observation revealed many characteristics of the colony formation of bacteria, filamentous structures of actinomycetes and fungi, and arrangement of the microbial cells with respect to the mineral soil constituents and of certain groups of organisms with respect to others. Slides which had been buried in soil for some time were also examined. This latter method was perfected by Cholodny (2), who first brought it to the attention of most scientists and introduced convincing evidence of its value by photographs clearly showing many typical bacterial colonies and characteristics of the growth of fungi, actinomycetes, and protozoa. The microorganisms and soil particles adhere to the slide surface in a thin film, and, under the microscope, the stained organisms appear in sharp contrast to the unstained inanimate material. The method has been used successfully by Demeter and Mossel (9) to detect changes in the population of field soils in response to fertilizer treatment and plant growth; it has also been used by Conn (8) and by Jensen (13, 14) to follow the changes brought about under laboratory conditions as a result of adding various organic and inorganic substances to soils and of altering the soil reaction, temperature, and moisture content. Jensen (13) made use of the contact slides to determine the relative abundance of Gram-positive, Gram-negative, and acid-fast bacteria in soils. By the same method, Ziemiecka (61–63) noted changes in the nature of the predominating microorganisms in the soil during the course of decomposition of added organic materials. The method enabled Eaton, King, and Hope (10, 16) to demonstrate cases of parasitism of the cotton root-rot fungus in the soil. The contact slide method was also used by Joshi (15) to study the changes in the nitrogen-fixing bacteria in soil, and by Meyer (29) to determine the growth of pure and mixed cultures in sterile soil [see also (59)]. Kriuchkova (23) modified the method by adding films of various agar media to the surfaces of the slides before inserting them in the soil. The organisms which formed colonies could be isolated and studied. Cholodny expressed doubt that this method would be much more useful than the common agar plate (3, 4). All who have used the contact slide procedure agree that it is most helpful in obtaining accurate evidence of the morphological characteristics of the soil population, the aggregation of various organisms, and the influences of environmental factors on changes in abundance, types, and distribution of microorganisms (3, 4, 39).

Most of the evidence concerning localization of microorganisms about roots has been obtained indirectly, by plate counts or by similar procedures. In relatively few instances have microscopic observations been made on roots of plants other than legumes and even in this case only with regard to the nodule bacteria. Zycha (64) planted pea seeds in a mineral agar medium that was inoculated with the pea bacterium, which formed a mantle of cells about

the roots even to the bottom of the tubes. The bacterial development was apparently supported by materials coming from the roots, since no such growth took place about glass rods which were inserted in other tubes of agar. Similar results have been obtained in this laboratory. While some legumes were growing in agar in large glass tubes, a vetch culture became contaminated with a black yeast-like fungus. This organism did not appear to affect plant growth even though it developed abundantly about the roots. It did not make visible growth elsewhere in the agar medium but appeared to be favored in the region of root growth. There is, of course, no assurance that the roots would react similarly in soils.

Rossi obtained fragments of roots and root hairs on his slides prepared from soils, and he reported that there were "no static or numerical relations between the clusters and the absorption apparatus of the plants, but the clusters were concentrated about the roots" (40, p. 65). Since he states that clusters represent resting cells, one would assume that he did not believe that roots appreciably affected microbial development.

Direct microscopic examination of roots disclosed a much more intimate association with microorganisms. Thom and Humfeld (52, 53) reported that the epidermal cells and cortical parenchyma of healthy roots were infested with mold hyphae; bacterial cells were abundant, filling some of the plant cells and being absent or present in small numbers in others. Invasion of the lumen of root hairs by hyphae was not uncommon; ". . . each rootlet and each root hair is fringed with microorganisms. . . . All around these complex branching systems swarms of microorganisms line every pore of the soil" (51, p. 57–58).

The contact slide method was used by Hulpoi (11) in much the same manner as that in the experiments to be presented. He placed cover glasses in soil in which plants were grown. The plant roots passed over the glasses, and some became attached. After these preparations were stained, the influence of the roots on microbial development could be detected by microscopic observation. Photomicrographs illustrated localization of rod-shaped bacteria on or in root hairs of oats and lupines; in general, the bacterial cells were scattered, but in other cases they developed in dense aggregates on the root hairs. The evidence emphasizes the condition described by Thom.

EXPERIMENTAL METHODS

The contact slide method was used in much the same way as that described by Cholodny (2). Microscope slides were inserted in soil in the field, plants were grown in the soil, and the slides were periodically removed and examined. The soil is classified as Sassafras loam and varied in reaction between pH 6.0 and 6.8 during the season. Slides twice the usual size (52 by 77 mm.) were placed in the soil in a vertical position. Seeds or seedlings were then planted 1 to 3 inches above the slides. Seeds of the following plants were used: mangel beet, barley, maize, rape, vetch, and soybean. Pepper and tomato

seedlings were also planted. A portion of the field was kept free from all vegetation, and slides from this region served as controls to indicate the nature of the soil population in the absence of root development.

It was anticipated that as the plants developed, some of the roots would pass over the surfaces of the slides and that some portion would adhere to the surfaces. Roots did not appear, however, on all the slides. After the slides were taken from the soil one side of the slide was cleaned, the larger soil particles were removed from the other side, and the slides were dried and then stained with phenolic rose bengal (8) for 10 minutes. During the staining period the slides were kept warm over boiling water. After being washed and dried, the preparations were examined under the microscope, a 2-mm. apochromatic objective (n.a. 1.30) and 12.5 x compensating oculars being used. The immersion oil was added directly to the preparation without use of a cover glass. The entire surface of each slide was carefully explored, the abundance of microbial cells, characteristic formations, and particularly the relationships of the organisms to roots and organic detritus being noted. The slides were taken from the soil during the summer of 1934 and have been examined at various intervals during the last 3 years. Representative formations were photographed. At first a Reichert camera with which photomicrographs could be obtained only up to magnifications of 650x was used. Most of the photomicrographs, however, were made with a Bausch and Lomb Type H camera, a magnification of 1200x being obtained on the film. None of the illustrations in this paper have been retouched, and all are presented as obtained from contact prints.

NATURE OF THE SOIL POPULATION

On the slides from fallow soil or on portions of the other slides where roots had apparently exerted no influence somewhat limited microbial substance occurred, but diverse types of organisms were encountered. The organisms were more or less well distributed as colonies, the individuals and their arrangement suggesting orderliness.

Bacteria

Many of the bacteria occurred as isolated cells; some were coccoid, and others were thin or thick rods or spindle-shaped cells. There was no lack of scattered isolated cells. Much more prominent were the small aggregates of bacterial cells. The predominating forms were small coccoid or spherical cells, although a few larger ones were seen. Some of the larger cells, most of which were deeply stained, formed tetrads or packets; many of the pairs of cells were bean-shaped (fig. 1). Most of the cells were in compact aggregates and seemed to be imbedded in material which retained some of the stain. Many of the aggregates were associated with soil material. Colonies of these organisms were considered by Winogradsky to be typical representatives of his soils (60). Photographs by Romell (38, pl. 1A), by Demeter and Mossel

(9, fig. 2b), and by Koffman (17, fig. 21) show cells of somewhat similar appearance. Although many such organisms were seen on virtually all the slides, they were not the most common forms. Larger spherical cells in packets imbedded in lightly staining microbial substance (fig. 2) also occurred. They are considerably larger than most of the bacteria that were encountered, and it is possible that they are not bacteria.

The bacteria seen in greatest abundance were small, nearly spherical cells, commonly encountered in thin spreading aggregates or in denser groups rather variable in size; although some of the colonies were larger than the one shown in figure 3, most of them were small. Compact cyst-like masses, like the one in figure 4, would probably be typical of the clusters described by Rossi as inactive organisms (40).

Some idea of the abundance of these colonies of small coccoid cells can be gathered from the fact that about 300 such colonies were recorded on a single slide from the fallow soil even though no attempt was made to record all the colonies. These small coccoid cells in small and large aggregates were generally located in the midst of some mineral or organic material. They are not readily distinguished in photographs, since relatively few cells are in focus at any one time and the soil material obscures the details. As with all of the preparations, cells which are clearly distinguished by the ruby-red color lose much of their striking appearance in the black and white photographs.

An explanation for the predominance of cells of coccoid shape was advanced by Conn (7), who believed that the bacteria assume the nearly spherical shape under conditions of low nutrient level in the soil environment and that they become longer rods at times when they have access to readily available food material. The relatively scant microbial development in fallow soil and the arrangement of the cells further emphasize the fact that the fallow soil is poor in microbial food. A great change occurs where plants are growing and root parts become attacked, as will be discussed later.

Other cells, shown in figure 5, appeared to be cocci in tetrad formation but were actually rod-shaped cells having deeply stained ends; few organisms of this type were noticed. Larger rod-shaped bacteria were generally associated with decomposing bits of organic matter. Many thin, spreading, veil-like films of bacilli were encountered, however, without any evidence of organic materials undergoing decomposition nearby (fig. 6). Some of these colonies were very large and spread over an area equal to several of the microscope fields. A similar formation is shown by Cholodny (2, fig. 16). A few loose colonies were seen composed of fairly long cells tapering to points at the ends (fig. 7).

Cells like those shown in figure 1 suggest the appearance of Azotobacter, but pairs of spherical cells shown in figure 8 and particularly the colony of fairly large unevenly stained cells in figure 9 [see (2, fig. 18)] are more typical of this organism. Although such cells have been seen on many of the slides, they were not typically associated with any organic substances, nor

were they abundant enough to suggest that they were particularly active; several colonies of these cells were seen about rootlets.

Other colony types and bacterial shapes were encountered, but in such small numbers as to suggest that relatively few of them were present in the soil under consideration. Few spore-forming rods were seen (fig. 11); some other large rods, which may have been spore-formers, appeared. Two groups of long slender cells suggestive of the cellulose-decomposing bacteria of the genus Cytophaga were noted in the vicinity of root detritus on slides from maize (fig. 12). The cells stained well in the center and very lightly at the ends, which were pointed. Two groups of organisms of very unusual shape were seen on one of the slides from vetch. One of these colonies was composed of large, uniformly stained, vibrio-shaped cells (about 3μ long) pointed at the ends (fig. 10). Although they were somewhat larger than the common vibrios it is probable that they were bacteria. The other more unusual cells shown in figure 13 were lightly stained tubular rods more than 20μ long, having a deeply stained portion near one end which looked like a fairly large bacillus (about 2μ long). These cells are unlike any bacterial forms previously seen. On a slide from beets, a colony of fairly long rod-shaped cells occurred in palisade formation, much like those shown by Cholodny (2, fig. 16). A few typical sarcina packets of small cocci were noted.

It should not be concluded that the slides were covered with organisms, for the majority of slides had many more fields with no apparent microbial cells than fields showing microbial development.

Actinomycetes

Actinomycetes appeared in abundance on all slides, both in conidial forms and as unfragmented filaments, the conida being considerably more common. This agrees with Conn's observation made in 1918 that the conidia are so much more numerous than the filaments that undoubtedly virtually all the colonies of actinomycetes on the plates arise from the spores (6). It fails to support the contention of Lutman, Livingston, and Schmidt (27) that the organisms exist mainly in the soil as bits of mycelium with fewer spores. The differences of opinion are no doubt due to the difficulty in distinguishing conidia from bacterial cells in disturbed soil. It is readily understood from the large number of conidia and actinomycete filaments why such a large portion of the colonies on agar plates are formed by these organisms. As emphasized by others, the method more accurately portrays the true morphological characteristics of the actinomycetes in their relation to the soil environment than does any other method. The cells stain exceedingly well and disclose structures which are not readily detected by other means. The actinomycetes were generally stained better than any of the other organisms, but even with such fine preparations it was impossible to tell whether certain rod-shaped cells were bacteria or conidia of actinomycetes.

Many of the conidia were scattered and occurred in small aggregates of a

few cells or in short chains. Others, however, were encountered in large groups composed of many conidia including complete and broken spirals and fine nonfragmented filaments. Some of the aggregates of fragmented filaments are shown in figures 14, 15, and 16. In many instances the cell material was very profuse, covering an area of many fields of the microscope, thus indicating that there had been considerable decomposition of organic material. In figures 15 and 16, shadows of fine filaments appear in the background; it seems likely that they represent the vegetative mycelium of the actinomycetes, which is commonly finer than the fragmented aerial mycelium. Generally the actinomycetes were free of bacterial associates on the slides from the fallow soil and on those portions of the other slides where roots had not penetrated. The illustrations of bacterial colonies further emphasize the fact that very many, if not most, of the bacterial aggregates are also composed of single-cell types where readily decomposable organic matter is not present. Some associations of bacteria and actinomycetes were encountered, however, and occasionally both organisms developed profusely together. A very few actinomycete filaments were studded with bacterial cells, suggesting bacterial decomposition of the filaments.

In addition to the scattered conida, many branched mycelia bearing spring-like coils of fragmented filaments were encountered. Some of these formations are shown in figures 17, 18, and 21 (see also figure 57). Some of these coils were loose and open, and others were pressed closely together. Many of the spirals were broken apart, only remnants of the original formations being apparent. The coiled conidial filaments were coarser than the filaments from which they originated, and fragmentation was more readily seen in the coils. Other sporulating spirals were seen, the coils of which were in one plane, appearing like concentric circles of fragmented conidia. In figures 17 and 18 more than one type of fragmentation is apparent; most of the conidia are relatively close together, but others are well separated and appear to have constrictions between them. In figure 16 the conidia seem to be retained in a thin sheath; this is the type of spore connection most commonly observed. The spores pictured in figure 19 show no such connecting sheath but are held together by a thin strand. Whether the differences are typical for different species of actinomycetes or are indicative of different stages of maturity of the spores or whether they are brought about during the staining of the slides is not known.

The loose open growth of the colonies of the actinomycetes, even though spreading over a large number of microscope fields in some cases, re-emphasizes the fact that the soil medium is comparatively deficient in readily available nutrients.

An unusual type of formation was encountered in several places on one of the slides from the vetch soil (fig. 20). From a group of deeply stained small coccoid bodies, fine branched filaments radiated. It seems most likely that these were germinated spores of an actinomycete showing the early stage of

mycelial development. Many ropelike coils of actinomycete filaments like those shown by Demeter and Mossel (9, fig. 11a) and by Jensen (13, fig. 9) were also seen.

Filamentous fungi

Fungus filaments occurred in considerable abundance even on the slides from the fallow soil; many extended in all directions and were visible without the aid of the microscope. They were generally somewhat lightly stained, ribbonlike threads, but many short or long pieces of large, unstained, brown, septate mycelium were also seen. A great variety of spores occurred which were prominent by reason of their large size in comparison to the cells of the bacteria and actinomycetes. Among those most frequently observed were somewhat distorted nearly spherical spores about 2μ in diameter, belonging probably to the Fungi Imperfecti; a few scattered spores are shown in figures 63 and 64. Fusarium spores shown in figure 22 appeared singly and in large groups. Spores like those in figure 29 were seen more or less frequently on all of the slides. They were large cells (more than 20μ in length) containing four deeply stained bodies within the almost clear elongated envelope; a fifth dense body at one end protruded and undoubtedly was the point of attachment to the mycelium. They were probably spores of Helminthosporium or of a closely related genus. Dark brown unstained multicellular spores characteristic of Alternaria and related fungi were seen, and also a few dark brown, two-celled, egg-shaped spores like those in figure 30. The spores shown in figure 23 were virtually the only cells which did not remain in place on the slides covered with immersion oil. They floated in the oil and became dispersed. They were flattened circular spores having numerous striations which gave them a cogwheel appearance in one position. They were hollow on one side, and the general shape closely resembled that of the cap of an acorn. The unusual cells shown in figure 24 are also believed to be fungus spores. They were shaped like six-pointed stars with a deeply staining central body. Many other spores were seen, including large oval cells, elongated rod-shaped cells, spheres, and irregularly shaped cells which may have been distorted by the staining procedure; some of these stained deeply, and others were unstained brown spores.

Although almost all the spores were scattered, not many being attached to the sporangia, a few sporulating bodies were seen. The whorls of sterigmata in figure 25 characterize this as a Penicillium. Figure 26 shows a strange sporulating body. Several such structures arose from the same mycelial strand at distances of about 75μ apart. A bicellular or multicellular swelling occurred part way up the sporulating hypha; above this was a smaller swelling, and above this, a large spindle-shaped body that looked like a Fusarium spore. A filament bearing relatively large, single pear-shaped spores on each of the short hyphae is shown in figure 27. This organism is probably closely related to the genus Sporotrichum. A sporangium typical of the Mucoraceae was

also seen. The rectangular vacuolated cells in figure 28 are probably related to Monilia or Oidium. This illustration also includes a large nearly spherical fungus spore with a small pointed projection.

As has been frequently emphasized, the contact slide method reveals an abundance of fungus filaments in the soil, leaving no doubt as to the vegetative development of fungi in soil even in the absence of appreciable amounts of readily decomposable organic matter.

Algae

The most frequently distinguished algal cells were diatoms. Bristol-Roach stated (1) that although diatoms occur rather commonly in the soil, there is a great preponderance of green algae, mainly of the unicellular forms, in temperate zones. Very few algae other than diatoms were detected during these observations, probably because the large soft cells of the green and blue-green algae became so deformed by the desiccation and staining procedure that their identity was not apparent. Siliceous skeletons of the diatoms, free from all protoplasm, were common. Probably all of the diatoms which were seen belong to the group of Pennatae. Only one of the forms, that shown in figure 31, was characteristic enough in appearance to permit identification; it is probably *Hantzschia amphioxys* which has been found frequently in the soil (58). The majority of the cells were shaped like those illustrated in figures 31, 32, and 33. Numerous striations and pits could be seen. The striations sometimes extended over the entire surface of the skeletons as with the forms in figures 31 and 32. On other cells, such as those shown in figures 33 and 35, the striations seemed to be confined to the ends and ran diagonally toward the center of the skeleton. There were also various other forms, including the one in figure 34, spindle-shaped cells resembling Navicula, and cells considerably longer and more slender than any of those illustrated. Occasionally they were present in pairs or in small aggregates, but generally they were solitary. Shrunken protoplasmic contents can be seen in the forms pictured in figures 32, 34, and 35. Illustrations of diatoms obtained from soil preparations are shown by Wang and Chia (59), Koffman (18), and Demeter and Mossel (9). Some of their specimens resemble those mentioned in the foregoing description.

One chain of rather large nearly spherical cells was noted (fig. 36). Each cell contained several deeply stained particles located against the cell membrane. Their appearance suggests blue-green algae such as Nostoc.

Protozoa

No material was seen which was identified definitely as a protozoan. Undoubtedly many of the deeply stained, large, irregular bodies which were observed were trophic stages of protozoa; some of the dense spherical and similarly shaped cells were probably cysts.

Invertebrates

On each of the slides were skeletal remnants of some small animals, those most commonly seen being thin striated scales with an attachment point at one end, and thin tapering spiny setae (fig. 37). These materials were generally scattered but occurred in many places in large masses, each of which looked like most of the chitinous covering of a tiny animal; frequently claws were detected at one end of an elongated group of setae. The scales varied considerably in size. Some of the setae were smooth, and some were covered with spines; some were hollow, and others appeared to be solid.

Occasionally some filaments of fungi or actinomycetes appeared about these materials, but more commonly there was no evidence of microbial development about these resistant organic substances. There was more apparent microbial attack of a small animal shown in figure 38. Legs and body parts were still well preserved, but attached to them and radiating in all directions over many fields of the microscope was a fungus bearing single spherical spores on short slender hyphae. The fungus is probably a member of the Dematiaceae. The fungus development illustrates the localization of microorganisms in regions where food material is available. In such cases, however, the fungus may be responsible for the development of other organisms at some distance from the location of the animal, since its mycelium, which spreads for some distance, eventually would be attacked by bacteria or other microorganisms.

Nematodes were seen in only three places. A portion of one showing the head and the upper part of the body can be observed in figure 66; this nematode was in contact with a small root. The forms encountered were from 200 to 400μ in length.

Localization of microorganisms about organic detritus

The arrangement of the microorganisms with regard to other soil materials on the slides has repeatedly emphasized the fact that microbial development is extremely localized. Cells grow only where there is food, and large accumulations of cells are to be found only where there has been an abundant supply of such food. Humfeld and Smith (12) found that although bacteria were extremely numerous throughout a mass of green manure undergoing decomposition in the soil, the effects of the manure were very local, and the abundance of bacteria was not greatly modified a short distance away. Similar localization about organic materials was observed by Krassilnikov (20). Localization of a fungus about a small animal has been mentioned. Aggregates of bacteria and actinomycetes were very frequently encountered about bits of organic matter. Figures 39 and 40 are representative of this condition, showing considerable numbers of small rod-shaped and coccoid bacterial cells about and on the organic material. An extremely extensive dense colony of longer and larger bacilli is illustrated in part in figure 41. This

colony occurred on a slide from the vetch soil. It spread over 15 to 20 fields of the microscope, disclosing scattered bacterial cells and occasionally some septate fungus mycelium, shown in the picture, and actinomycete filaments. This was the largest mass of bacterial cells encountered at any one place on the slides.

The predominating bacteria in the colonies about organic matter were small coccoid cells; longer rods such as those shown in figure 41 were less common, and spore-forming rods were very rare. Groups of fungus spores were detected in some of these regions of active decomposition.

Localization of microorganisms about fungus filaments

Even more striking than the localization of microorganisms about organic detritus was the very common and extensive development of bacteria about fungus filaments (2, 8, 9, 13, 61, 62). Mycelium was abundant on most of the slides, and a considerable number of bacteria were in close contact with much of it. Such bacterial-fungus associations were not seen with the brown mycelium. The bacterial cells were most commonly scattered along the hyphae or in small aggregates (fig. 42). Some of the filaments were studded with small uniformly sized colonies (fig. 45). Many of the bacterial aggregates were large and dense (fig. 43), being composed of hundreds of tiny, lightly stained, coccoid cells difficult to resolve in photographs (fig. 44, 51). These large groups were particularly numerous on slides from the soils in which rape and vetch were growing. The small coccoid bacteria were by far the most common fungus associates, although occasional larger rod-shaped cells like those in figure 46 were seen. There is little doubt that these formations represent stages in the destruction of the filaments by the bacteria. This is emphasized by the fact that the filaments took very little stain, indicating the absence of appreciable amounts of protoplasm. One may be justified, however, in questioning how masses of bacterial cells, which seem to have considerably greater volume than the fungus filaments in the immediate vicinity, could have grown from the protoplasm of these filaments alone. It seems necessary to assume that they grew in part from products of the fungus growth; at least some of the latter must have been transported from other portions of the mycelium.

Fungus mycelium thus seems to be very susceptible to bacterial attack and probably does not generally persist for long in the soil, having at least shorter existence than many of the bacteria and actinomycetes. Certain environmental conditions may lead to rapid development of fungus mycelium which will soon be destroyed following exhaustion of the limited food supply or a change in the environmental conditions. This would explain in part the reason why, in response to certain soil treatments, comparatively little of the anticipated increase in abundance of fungi is detected by the plate method. Jensen (14) concluded that the contact slides were more satisfactory than plate counts for determining the density of fungus mycelium in the soil.

Localization of microorganisms about roots

The slides from soils supporting plant growth showed not only all the qualitative characteristics apparent on the slides from the fallow soil but also numerous additional ones. Quantitatively the population was much more dense even where there was no evidence of root material in the immediate vicinity on the slide.

Slides free from roots were characterized by well-defined and almost neat development and regular scattering of various colonies and occasional cells. In the presence of root material the picture was greatly altered. There were many irregular aggregates of great numbers of various organisms indicating rapid and extensive cell development, typical of what Thom (50) refers to as the "explosive" type of growth. Here may be found bacteria, actinomycetes, and fungi developing together in a confused arrangement. The condition may persist for a considerable distance from the location of the apparent root material, although most of the cells are confined to the roots. The greater the apparent degeneration of the root parts, the greater is the variety of the microbial invaders. Considered as a whole, the slides from planted soil supported much more profuse development of microorganisms than did slides from fallow soil.

The organisms are more readily illustrated about root hairs than in contact with larger roots, since the root hairs are generally lightly stained and reveal the more deeply stained microbial cells. Some typical root hair formations are shown in figures 47–50, 52–56, 58 and 60, some of which are similar to Hulpoi's illustrations (11). The ribbon-like root hairs support occasional fairly large rods, but more commonly very tiny coccoid cells are found, many of which are in chains either within the root hairs (figs. 47, 48, 52) or spread over the surfaces and radiating from them (figs. 53, 54). Most of the hundreds of root hairs attached to the small vetch root from which figures 48 and 54 were obtained were permeated with chains of these cells. These threads of tiny cells resemble actinomycetes in many respects, particularly since some of them appear to be branched (figs. 48, 61, 62). The filaments are smaller, however, than most of the actinomycete conidia. Furthermore, previous results (48) indicated that actinomycetes are not very much more abundant about roots than in soil free from roots. If such cells as these are actinomycetes, and if they invade roots as generally as these microscopic studies indicate, much greater numbers of actinomycete colonies should have been obtained on the plates from the root samples. It seems most likely that these are chains of small bacterial cells.

On some root hairs the bacterial cells were scattered and occurred in relatively small numbers. Occasional small colonies (fig. 58) were seen. Many chains of bacterial cells developed in close contact with the exterior of the root hairs, giving the edges a beaded appearance (figs. 47, 53, 58). In figure 49 the bacteria are shown in the form of a mantle about the tip of a root hair.

Large numbers of bacterial cells were localized about a root and root hairs shown in figures 55 and 56, some of the cells being scattered on the root surfaces and some forming colonies near the roots. In figure 59 groups of bacteria are shown about a clear zone which was probably occupied by a root hair or small root which became dislodged during the preparation of the slide.

It is not possible to state definitely whether or not the root hairs were in a vigorous condition at the time the photographs were made. In many regions of microbial development it was difficult to determine whether the material undergoing decomposition consisted of dead roots, sloughed-off cells, dead root hairs, or root excretions. Many of the root hairs, however, were followed from their point of attachment to the small roots throughout their entire length, and they appeared to be intact except for the microbial associates. Many of them showed deeply stained portions indicating the presence of considerable amounts of protoplasmic material (figs. 47, 48, 49, 54, 58, 60). Microorganisms were not detected on all the root hairs; however, their common occurrence seems to justify the conclusion that bacteria develop in abundance upon apparently vigorous root hairs. The attack of the latter by microorganisms is so rapid that it is unlikely that appreciable residues persist long after death of these root parts. Large numbers of bacteria were also seen on small roots.

Microbial development was not confined to the bacteria. Relatively large, branched, septate mycelium is quite evident in figure 48. Fungus mycelium was in evidence about many roots but not in such great abundance as were the bacterial cells. Well-defined development of actinomycetes was more apparent than that of fungus mycelium. Short conidial branches, unfragmented filaments, and aggregates of conidia were seen about the root hairs (fig. 60) but in small amounts compared to the cell material of the bacteria. The actinomycete shown in figure 57 appears to have grown from a small root. The extensive mycelium which spread over many fields of the microscope indicates that considerable decomposition of organic matter had occurred. The coiled sporulating hyphae clearly illustrate the characteristic morphology of the soil actinomycetes. The fragmented conidia shown upon the root hair in figure 50 might easily be confused with bacterial cells.

Considerable evidence of more advanced microbial attack of root hairs was discovered, particularly on slides which had remained in the soil for several weeks, some of the preparations appearing much the same as the pictures by Hulpoi (11, figs. 4 and 5). Figures 61, 62, 63, 64, and 66 are typical of this decomposition of roots. The root hairs in figures 61, 63, and 64 are shadowy in outline and are spotted with bacterial cells, actinomycete conidia, and fungus spores. Filaments of actinomycetes form connecting networks between many of the root hairs. About some of the microbial aggregates there were residual root parts, and in some places only masses of bacterial cells and filaments of actinomycetes and fungi remained where the roots had

been located. Bacteria and actinomycete filaments can be seen in figure 66 in the vicinity of a larger root; the dark projection with a lighter end is the head part of a nematode, the sinuous body of which was in close contact with the root. A portion of a relatively large root is shown in figure 62. Much of the organic material had been decomposed, and in its place appeared chains of tiny coccoid cells forming a profuse network. They have much the same appearance as the chains of cells commonly encountered in the root hairs (figs. 47, 48, 50, 52, 53, 54). Great numbers of larger rod-shaped bacterial cells were also present in other regions of this decomposing root, and masses of bacterial cells as well as filaments of actinomycetes and fungi radiated from it in various directions. In figure 65 small coccoid cells appeared in a mass, projecting from, as well as at a short distance from, a large root where a small root may have been decomposed.

Some roots of vetch were completely covered with masses of bacterial cells. They stained so deeply that it was difficult to obtain a good reproduction of the individual cells which composed the aggregates (figs. 67, 68). There were even swellings upon the roots where the organisms had accumulated in large numbers. Actinomycete filaments radiated from these roots; some fragmentation is evident, and a well developed spiral can be seen in figure 68. There is little doubt that these roots were almost completely decomposed, since only short portions could be found on the slides and very little root material remained, the root outline being preserved principally by the microbial cells.

Specific influences of the various plants

There was very little evidence of differences in the response of the microorganisms to different plants. Although the extent of microbial development was not the same on all slides, it seemed to be determined more by the abundance of root residues on the slides than by the type of plant growing in the soil. The slides from vetch consistently had large numbers of organisms, but one of the slides from the beet soil had the most profuse microbial development of all the slides examined. Only vetch roots were found completely covered with bacterial cells in the manner shown in figures 67 and 68. Fungus filaments were particularly abundant on some of the slides from the rape soil. There is little justification, however, for concluding that such conditions are specific for these plants. More closely controlled conditions and more numerous observations are needed to obtain information as to the specific organisms which are particularly favored by any one plant and as to the characteristic root formations of any plant.

Likewise there were no striking differences in the appearance of the slides which were removed from the soil at various periods after the planting date. No attempt was made to determine the conditions about roots of very young plants. The first slide was taken from the soil 23 days after planting (June 28), and the slide which remained in the soil for the longest period was re-

moved 128 days from the time of planting. After the slides had remained in the soil for several weeks, they showed more root material and associated organisms and considerably more roots in somewhat advanced stages of decomposition than did the slides which were first removed. Otherwise, and except for differences in the relative abundance of microbial cells, the slides had much the same microscopic appearance, irrespective of the age of the plants when the slides were removed from the soil. Some differences undoubtedly could have been obtained by varying the experimental procedure.

DISCUSSION

Although there is considerable microbial development in contact with intact roots, a large part of the root hairs and surface of larger roots appeared to be free from microorganisms. By far the greatest microbial development seems to be at the expense of dead root parts. Most of the organisms are unquestionably saprophytes concerned in the decomposition of root residues, but some of the organisms may be of more significance in the development of plant roots. Thornton (54, 55) obtained evidence that some substance is excreted from the roots of legumes which stimulates infection of the root hairs by the nodule bacteria. Ludwig and Allison (26) observed that maize growing in association with the legumes also favored legume nodulation, presumably through some organic materials originating from the maize roots. Virtanen has found that large amounts of organic nitrogenous materials are liberated from the roots of inoculated legumes [(57) see also (31)]. In a recent review, Loehwing recorded considerable evidence of the excretion of both organic and inorganic materials from roots (25). The ability of certain legumes and other plants to absorb phosphorus from relatively unavailable materials (25, 35, 36) has been ascribed to the excretion of organic acids from the plant roots (30, 42, 43). It thus seems likely that the development of microorganisms on roots might be brought about in part by organic excretions which the organisms use as food material.

Sherman and Hodge (44) found that at least some plants contain material having bactericidal properties. If this is a condition common to all plants, microbial development within intact tissues should be rare except in cases of parasitism or such special cases as nodule development by the legume bacteria (48).

Other factors which are concerned with the development of microorganisms about and within roots have been discussed previously (41, 45, 48).

The microorganisms which grow in the rhizosphere also affect growth of higher plants. In addition to the effects of the common products of microbial development (45, 48), certain organic substances (hormones, vitamins, plant stimulants) may be produced by microorganisms and affect the rate of plant maturity, cell growth, and root formation (24). Niethammer found that some of the common soil fungi favored germination of seeds and seedling development in sterilized soil and in agar medium (32).

It was not surprising that cells resembling Azotobacter were encountered somewhat infrequently and in very small numbers in comparison with the total number of bacteria. Although it is logical to suppose that the rhizosphere is a favorable region for development of the nitrogen-fixing bacteria (48), and even though Azotobacter has been recovered from plant roots (33, 34), in no case has it been demonstrated that these organisms are present in sufficient abundance to be of particular significance in the growth of higher plants. The claim of some Russian investigators (Kostytschew, Sheloumova, and others) that plant growth is improved by inoculating the soil with Azotobacter is not completely convincing (24). Krassilnikov concluded that the rhizosphere of maize and wheat does not favor the development of Azotobacter but that such bacteria as *B. denitrificans* and *B. fluorescens* grow well about roots of these plants (19). Since he used solution cultures, his results may not be typical of what occurs in the soil.

Although the amount of bacterial cell substance appeared to be greater than the amount of any other microbial material, conidia and filaments of actinomycetes were very numerous and at times more abundant than the bacterial cells. Conn reported some years ago that actinomycetes are particularly numerous in sod land and are active in the decomposition of grass roots (5). The present results indicate that actinomycetes are to be found in abundance about root materials of many plants and are commonly encountered, even though less abundantly, throughout the soil even in the absence of root development. Ziemiecka (61, 62) classified actinomycetes as secondary organisms which follow either bacteria or fungi in the decomposition of organic materials. No exact information concerning this point has been derived from the root studies, but, since cells of actinomycetes are commonly encountered even where there is evidence of decomposition in the absence of other organisms, it is unlikely that actinomycetes are active solely in advanced stages of decomposition.

It is not possible to make an accurate estimation of the relative abundance of fungus material; although the filaments and spores are much less frequently encountered than are the cells of bacteria and actinomycetes, the amount of cell material represented by a single fungal hypha would be as great as that contained in numerous bacterial cells. It is quite likely that in many places the fungus material exceeded the amount of substance in the cells of both the bacteria and actinomycetes. As has frequently been noted, numbers of organisms as estimated by plate counts give an erroneous impression of the significance of the various representatives of the soil population; this is particularly true with the filamentous fungi. From the fact that bacteria were commonly localized about fungal hyphae, it can be concluded that fungi were responsible for a considerable portion of the increase in numbers of bacteria in the rhizosphere.

It is still uncertain why very little influence of plants on abundance of fungi was detected by means of the plating method whereas the slide preparations

show a pronounced increase in fungus development under growing plants. It may be that the bacterial cells are viable for a longer period than is the fungus mycelium, or that the fungi produce relatively few spores, or that the media which were used for plating were not suitable for cultivation of the predominating soil fungi. The brief existence of fungal hyphae in the soil and the inadequacy of the plate method for determining the amount of mycelium are probably responsible for the lack of agreement in the results obtained by the two methods.

The illustrations included in this paper reproduce a few of the great number of varied formations which characterize the development of microorganisms in the soil. They show some of the most typical formations, those which were particularly obvious, and those which appeared to demonstrate interesting relationships between the microorganisms and the soil materials. Although they convey but a small portion of the complete picture of the arrangement of the microbial cells about the soil particles and the associations of various organisms, still they give some suggestion of the actual manner in which these microorganisms exist in their natural habitat.

The contact slide method has been extremely useful as a means of obtaining this evidence, particularly that concerned with the localization of the microorganisms about roots. The method provides a means of obtaining a more accurate conception of the development of microorganisms in response to root growth, the types of organisms affected, the localization of the individuals and groups, and the sequence of morphological types. However, it has certain limitations (3, 4, 13). It is unfortunate that there is no possibility of cultivating the organisms which are seen on the slides. Nothing can be ascertained concerning the physiology of the microorganisms which are encountered, and it is practically impossible to recognize specific bacteria on the basis of morphology alone. Furthermore, it is necessary to speculate as to the time the microorganisms developed upon the slide. When examining slides which have been in the soil for several weeks, it is not possible to state precisely when or under what conditions these organisms appeared. These obvious limitations, however, do not appreciably affect the value of the method for morphological studies. Certainly the present studies have made it possible to visualize more clearly some of the details of the root-microorganism associations which were known to exist from the results obtained by the plate method. The results emphasize the exactness with which Thom described the root conditions responsible for microbial development (50, p. 161):

> This bacterial distribution is directly dependent upon continual production of new epidermal cells, parenchymatic cells and root hairs at the growing root tip. The root-cap consists of soft walled parenchymatic cells which are constantly renewed from within and continuously dying and decomposing on the outside with remains of the outer cells crushed into the surrounding soil. Similarly the epidermal and cortical cells and the root hairs of the growing root itself, function actively only for a short time, after which they "slough off." This is readily seen under the microscope to mean that they are rotted by moulds and by countless numbers of bacteria.

SUMMARY

The buried slide technic was used to determine the nature of the development of microorganisms about roots of growing plants. The method proved to be useful for demonstrating some of the colony formations and growth characteristics of various soil microorganisms. Some of the organisms which developed in response to root growth and the types of microbial formations on root hairs were readily observed.

As had also been determined by the plate method, microbial development was found to be much more extensive about roots than elsewhere in the soil. The effect of roots is rather local, a large portion of the organisms developing in close contact with the roots and root hairs. The mycelium of the filamentous fungi may spread for some distance from the organic matter which is being decomposed. Small coccoid bacteria commonly appear in abundance on the fungus mycelium. Bacteria were found as scattered cells and in small aggregates about root hairs, conidial fragments and filaments of actinomycetes were recognized, and branched filaments and scattered spores of filamentous fungi were seen.

Microbial development was most extensive where dead root material was decomposing. In such regions rather large masses of cell material and many different organisms occurred in a confused arrangement, bacteria and actinomycetes predominating. The microorganisms were not confined to dead plant substance but occurred in considerable abundance about, upon, and within roots and root hairs that seemed to be in a vigorous condition.

The bacteria occurring in greatest abundance about the root hairs, fungus filaments, and decomposing organic matter were small, coccoid, lightly staining cells. Longer rods were detected, but spore-formers were seldom encountered.

No striking differences were noted in the types of microorganisms associated with different plants. The most apparent change in the population as the plants became older was the increase in abundance of organisms and the greater amount of decomposing root material.

In addition to the microorganism-root associations, many characteristic types of bacterial cells and colony types were seen, including small coccoid cells, Azotobacterlike cells, rods, fusiform organisms, and a large vibrio. Actinomycetes were represented mostly by fragmented conidia; springlike coils of sporulating filaments and branched vegetative mycelium were frequently encountered. Fungus mycelium was abundant, various types of fungus spores were seen, and sporangia of a few different fungi were noted. There were several types of diatoms, but no cells typical of protozoa were recognized. Chitinous remains of many small invertebrates were found.

Various microbial cells were encountered on the slides from the fallow soil, but the cells were less numerous and more uniformly distributed in colonies and in small scattered aggregates than where roots had penetrated.

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PLATE 1

FIG. 1. Small colony of relatively large bean-shaped bacterial cells. From rape; slide in soil for 126 days. 1200 ×

FIG. 2. Packet of large, nearly spherical bacterial (?) cells imbedded in capsular material. From vetch; slide in soil for 128 days. 1200 ×

FIG. 3. Very small coccoid bacterial cells in a loose colony. From maize; slide in soil for 40 days. 1200 ×

FIG. 4. Oval, compact, cystlike bacterial colony about soil material. From maize; slide in soil for 40 days. 1200 ×

FIG. 5. Loose colony of terminally staining bacilli about soil material. From vetch; slide in soil for 71 days. 1200 ×

FIG. 6. Large, spreading veillike colony of lightly staining bacilli. From vetch; slide in soil for 53 days. 1200 ×

FIG. 7. Loose colony of fairly long, spindle-shaped bacterial cells. From maize; slide in soil for 40 days. 1200 ×

FIG. 8. Spreading colony of fairly large spherical cells commonly in pairs (Azotobacter?). From fallow soil, 37 days after slides were inserted. 650 ×

FIG. 9. Compact circular colony of spherical cells, probably Azotobacter. From vetch; slide in soil for 128 days. 1200 ×

FIG. 10. Colony of large, uniformly stained, vibrio-shaped cells. From vetch; slide in soil for 53 days. 1200 ×


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PLATE 2

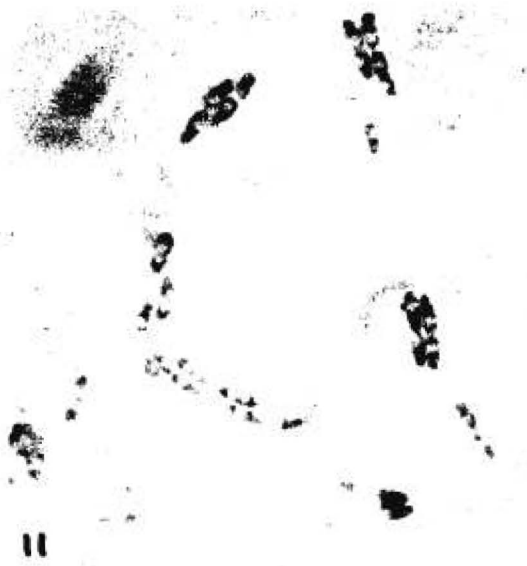
FIG. 11. Loose colony of large spore forming rods. From rape; slide in soil for 37 days. 1200 ×

FIG. 12. Long slender spindle-shaped cells with central portion stained more deeply than the ends. Located about organic material and spreading over several microscope fields. Resembles Cytophaga. From maize; slide in soil for 40 days. 1200 ×

FIG. 13. Very long, lightly stained, tubular cells, each with a deeply stained rod shaped body near one end (Bacteria?). From vetch; slide in soil for 53 days. 1200 ×

FIG. 14. Fragmented filaments of an actinomycete, showing conidia in short chains. Located near a bit of decomposing organic matter and spreading over several microscope fields. From rape; slide in soil for 37 days. 650 ×

FIG. 15. Fragmented actinomycete conidia. Deeply stained conidia and shadowy filaments of finer, lightly stained cells. Near decomposing organic matter. From rape; slide in soil for 37 days. 650 ×


11


14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12


13

PLATE 3

FIG. 16. Fragmented actinomycete filaments showing deeply stained conidia and shadowy outlines of finer, very lightly stained filaments. Portion of a very large group of cells spreading over many microscope fields in vicinity of decomposing organic matter. The conidia appear to be retained in a lightly stained sheath. From vetch; slide in soil for 53 days. 1200 ×

FIG. 17. Intact colony of actinomycetes showing branched filaments bearing open, spring like coils of conidia. The coil in the upper right hand section is composed of larger, more nearly spherical conidia than the others. From vetch; slide in soil for 53 days. 1200 ×

FIG. 18. Similar to figure 17. The loose coil near the top is composed of conidia which are nearly spherical and separated from one another. From vetch; slide in soil for 53 days. 1200 ×

FIG. 19. Chains of cells, believed to be actinomycete conidia, held together by thin strands. From maize; slide in soil for 40 days. 1200 ×

FIG. 20. Deeply stained germinated cells, believed to be actinomycete conidia, bearing fine branching filaments. From vetch; slide in soil for 53 days. 1200 ×

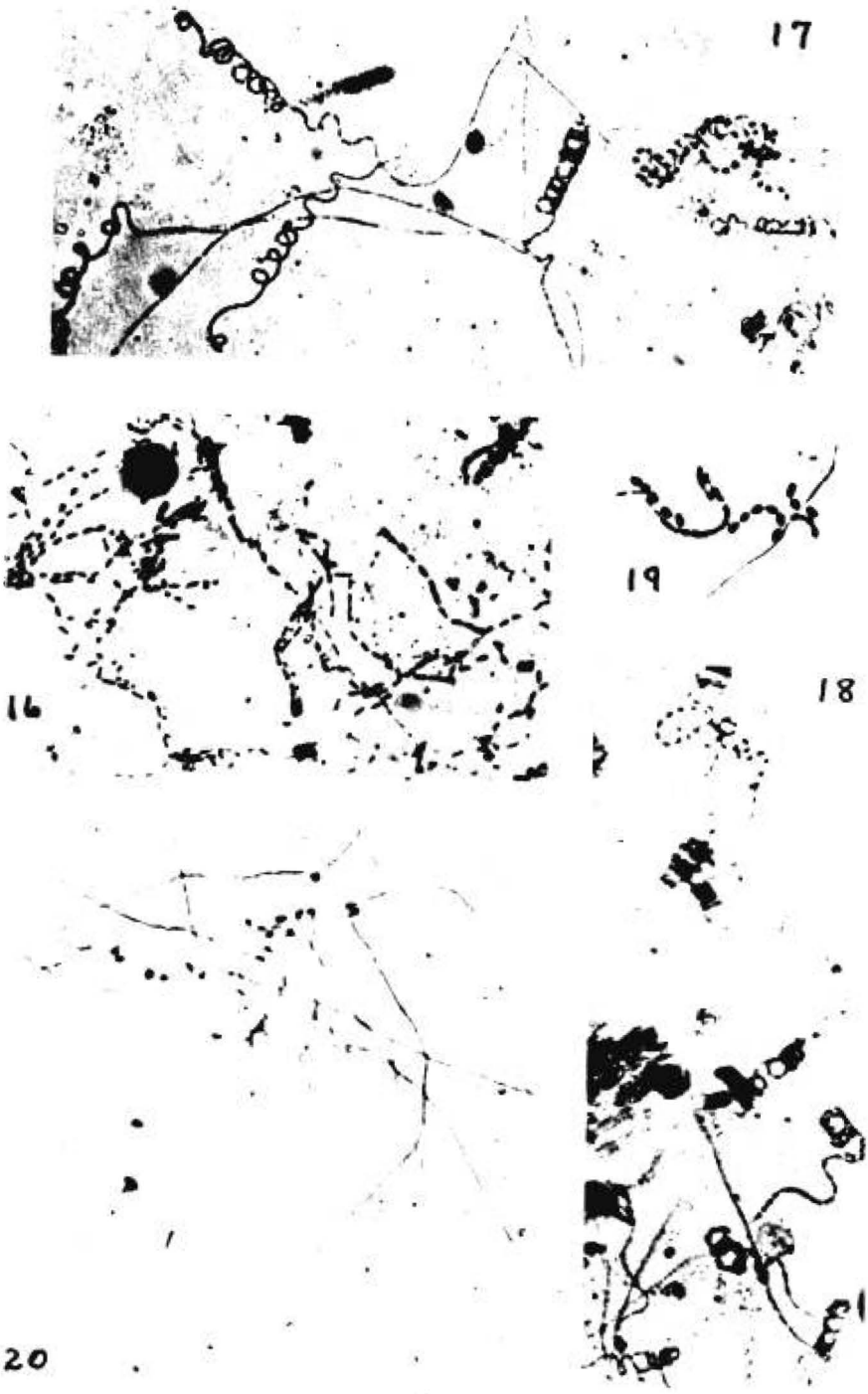
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PLATE 4

FIG. 21. Branched actinomycete filaments bearing open coils, some showing fragmentation into conidia. Similar to figures 17 and 18. From vetch; slide in soil for 53 days. 1200 ×

FIG. 22. Fungus spores, probably Fusarium. From fallow soil, 37 days after slide was inserted. 650 ×

FIG. 23. Cup-shaped brown fungus spores showing numerous striations. Shaped much like the cap to an acorn. From rape; slide in soil for 126 days. 1200 ×

FIG. 24. Star-shaped cells with deeply staining edge and central body (fungus spores?). From vetch; slide in soil for 128 days. 1200 ×

FIG. 25. Sporangium of a Penicillium showing whorl of sterigmata. From vetch; slide in soil for 71 days. 1200 ×

FIG. 26. Sporangium of an unidentified fungus. Several of these arose from the same mycelium nearby. From vetch; slide in soil for 37 days. 1200 ×

FIG. 27. Pear-shaped spores born on short slender hyphae. The fungus may be related to Sporotrichum. Many such sporangia arose from the same mycelium. From rape; slide in soil for 37 days. 650 ×

FIG. 28. Large rectangular, vacuolated cells probably either Oidium or Monilia. In lower center is a large, brown, nearly spherical fungus spore. From rape; slide in soil for 37 days. 650 ×

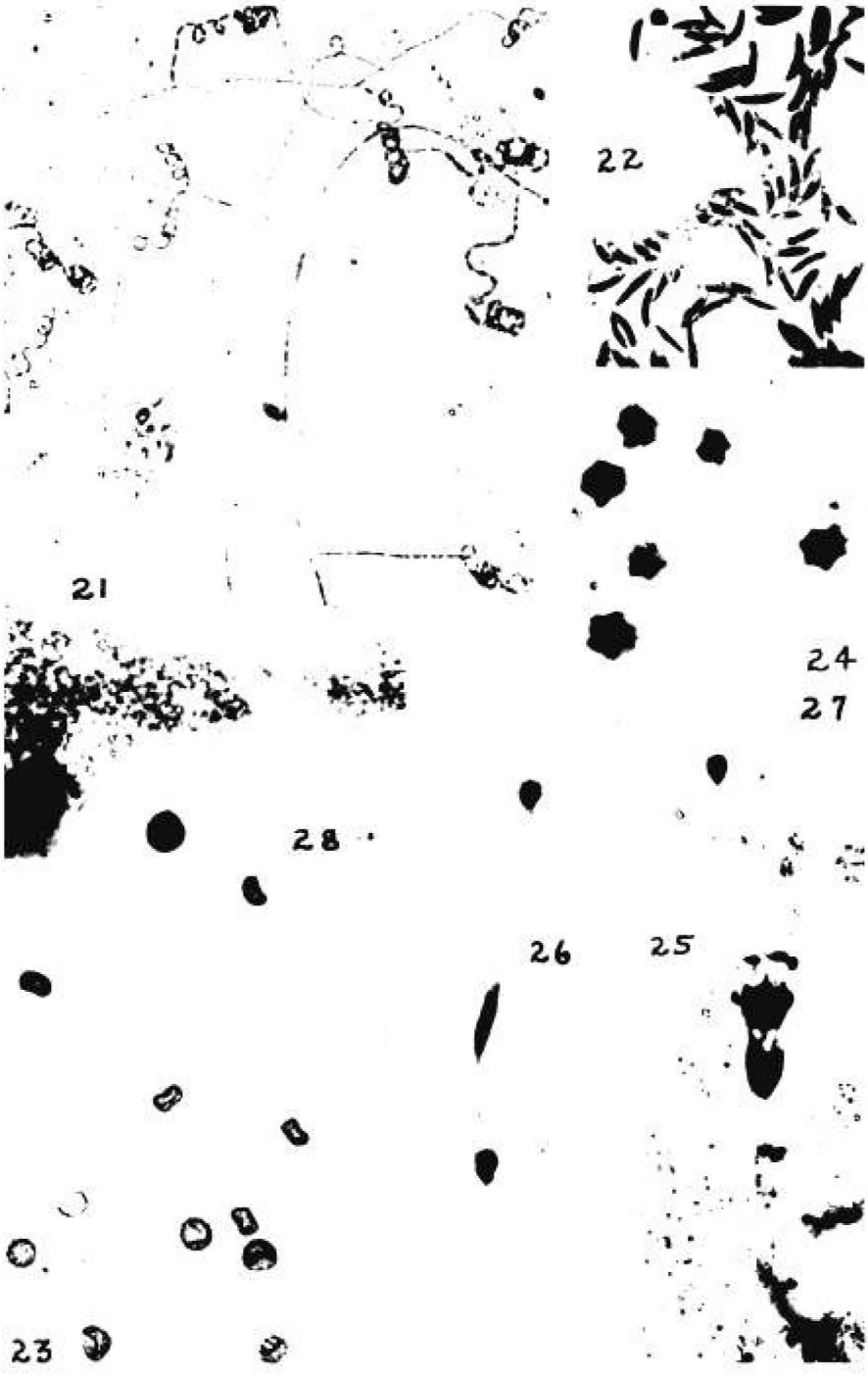
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PLATE 5

FIG. 29. Large fungus spores with five deeply staining bodies. Probably related to Helminthosporium. From rape; slide in soil for 37 days. 650 ×

FIG. 30. Dark brown, egg shaped fungus spore. From vetch; slide in soil for 37 days. 615 ×

FIG. 31. Diatom skeleton, probably *Hantzschia amphioxys*, with pitted upper border. From maize; slide in soil for 40 days. 1200 ×

FIG. 32. Elongated diatom with deeply stained protoplasmic residue near one end. Borders were pitted and numerous striations were apparent under the microscope. From vetch; slide in soil for 128 days. 1200 ×

FIG. 33. Small, thin diatom skeleton with constricted middle region. Striations near ends run diagonally toward the center from each side. From maize; slide in soil for 40 days. 1200 ×

FIG. 34. Diatom with wide pitted borders. Stained deeply over a large portion of the cell. From vetch; slide in soil for 128 days. 1200 ×

FIG. 35. Diatom showing ridged borders near both ends. Partly stained. From maize; slide in soil for 40 days. 1200 ×

FIG. 36. Chain of large spherical cells with deeply staining particles close to the cell walls (blue green alga?). From vetch; slide in soil for 128 days. 1200 ×

FIG. 37. Insect parts; thin striated scales and spiny setae. From rape; slide in soil for 37 days. 650 ×

FIG. 38. Small animal being attacked by a fungus bearing single spherical spores on short hyphae; probably one of the Dematiaceae. The fungus filaments spread for a long distance from this location. From fallow soil, 37 days after inserting slide. 300 ×

FIG. 39. Localization of large numbers of coccoid bacteria about a small root which is undergoing decomposition. From vetch; slide in soil for 71 days. 1200 ×

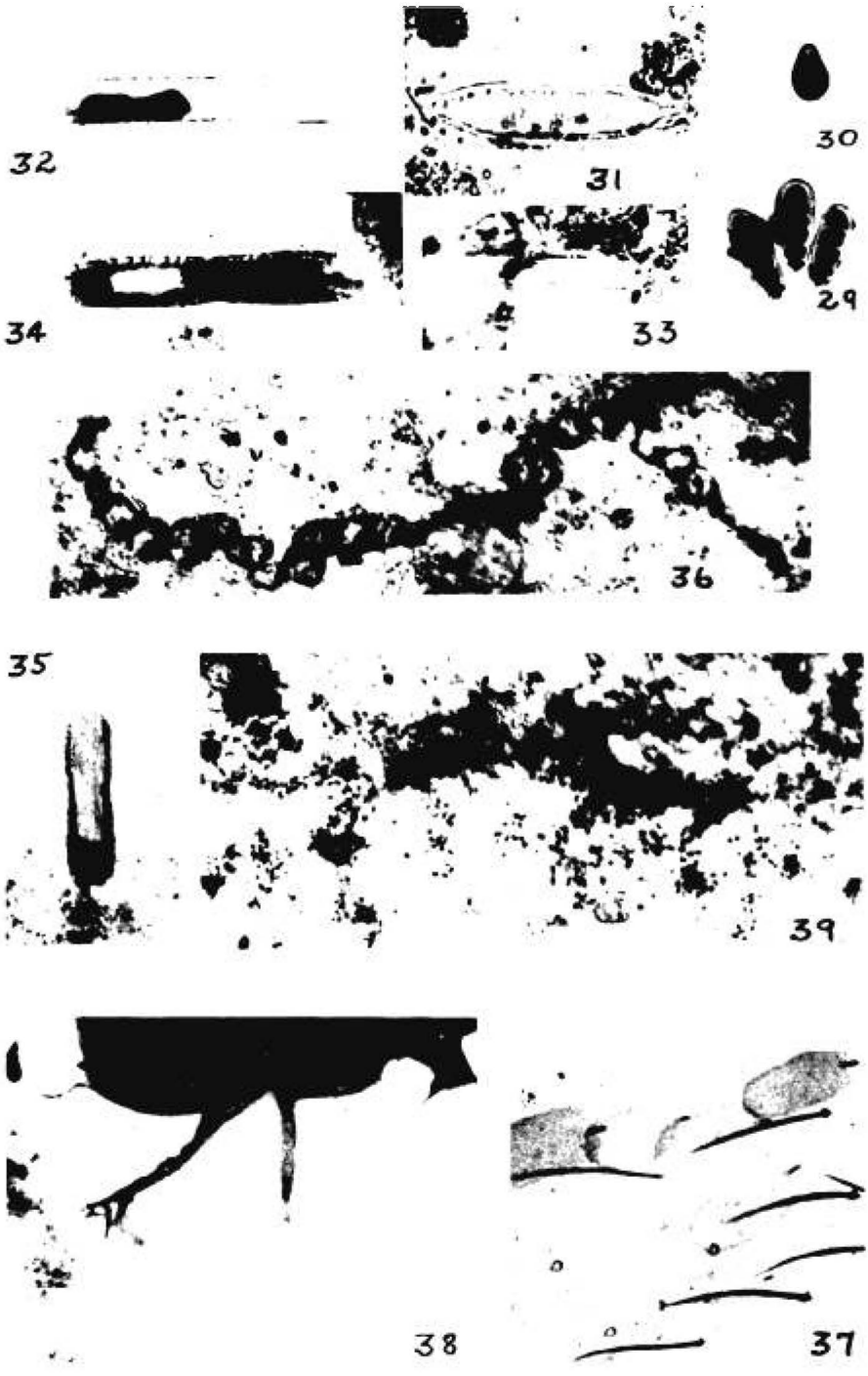
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PLATE 6

FIG. 40. Short rod shaped bacteria developing about and upon a piece of root material. From vetch; slide in soil for 71 days. 1200 ×

FIG. 41. Edge of a large dense colony of bacilli growing upon organic material. A branched fungus filament showing septation is also apparent. From vetch; slide in soil for 53 days. 1200 ×

FIG. 42. Scattered, small, coccoid, bacterial cells developing along a fungus filament. From vetch; slide in soil for 71 days. 1200 ×

FIG. 43. Aggregates of tiny, lightly stained, coccoid, bacterial cells developing about fungus mycelium. From rape; slide in soil for 37 days. 1200 ×

FIG. 44. Large dense colonies of tiny coccoid bacterial cells growing in contact with fungus mycelium. The individual bacterial cells are scarcely visible in these large colonies. From vetch; slide in soil for 37 days. 1200 ×

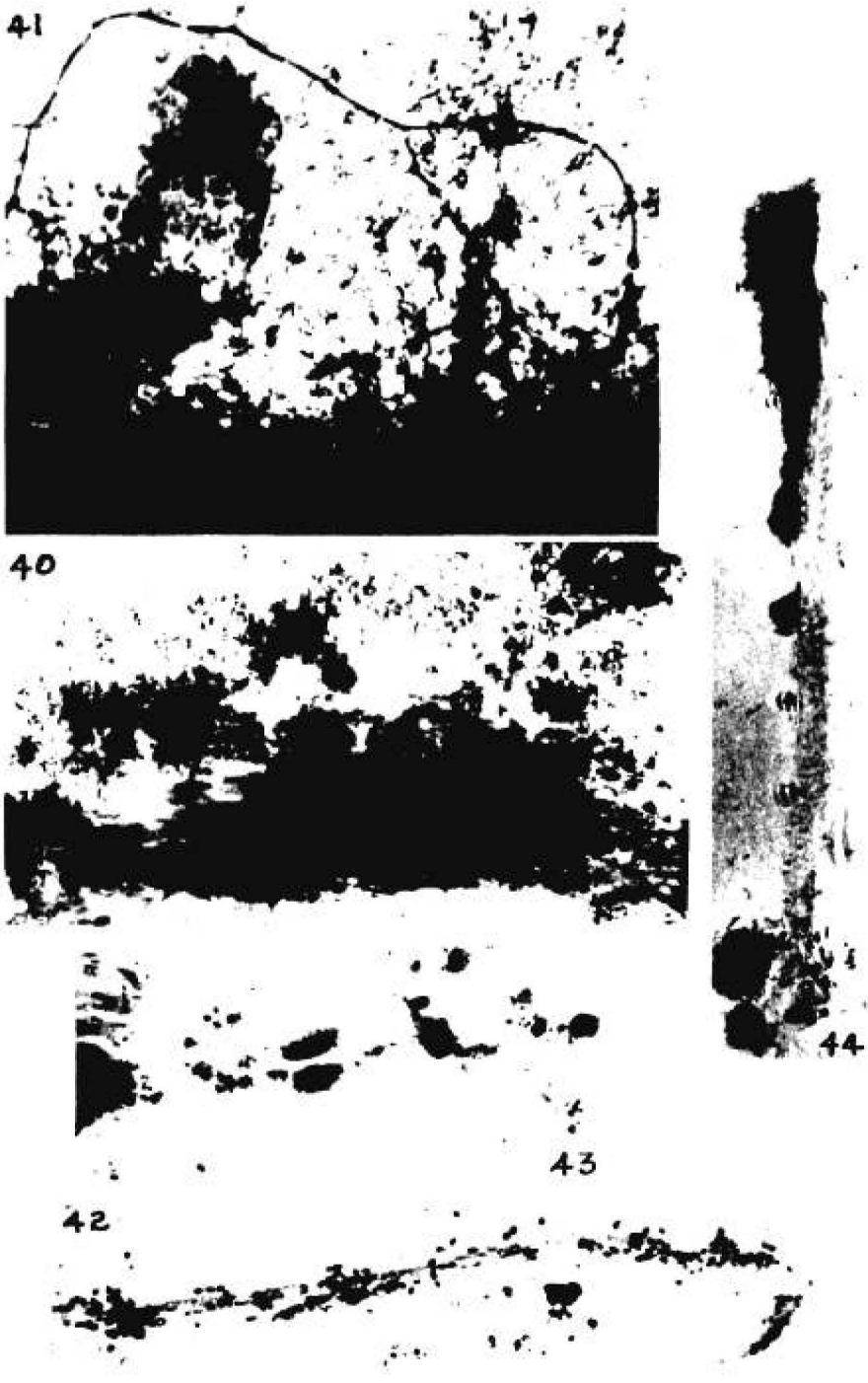
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PLATE 7

FIG. 45. Small colonies of coccoid bacterial cells growing along fungus mycelium. From vetch; slide in soil for 53 days. 1200 ×

FIG. 46. Considerable numbers of small, rod shaped bacteria developing in contact with lightly stained fungus filaments. From vetch; slide in soil for 71 days. 1200 ×

FIG. 47. Root hairs with chains of very small, rod shaped bacteria (upper center) and larger rods in contact with a root hair (bottom) and scattered at various other locations. From maize; slide in soil for 40 days. 1200 ×

FIG. 48. Ribbon like root hairs invaded by numerous chains of tiny coccoid cells, probably bacteria. Large, branched, septate, fungus mycelium also developing about the root hairs. From vetch; slide in soil for 53 days. 1200 ×

FIG. 49. Terminal portion of a root hair showing numerous short rod shaped bacteria in the form of a mantle. From mangel beet; slide in soil for 50 days. 1200 ×.

FIG. 50. Short chains of actinomycete conidia in contact with an almost colorless root hair. A few lightly stained fine filaments can also be seen. From rape; slide in soil for 126 days. 1200 ×


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PLATE 8

FIG. 51. Dense masses of tiny coccoid bacterial cells growing about a fungus filament. From barley; slide in soil for 53 days. 1200 ×

FIG. 52. Chains of small coccoid bacteria upon and within root hairs. From vetch; slide in soil for 71 days. 1200 ×

FIG. 53. Chains of small coccoid bacterial cells developing about root hairs. From rape; slide in soil for 126 days. 1200 ×

FIG. 54. A group of large ribbonlike root hairs supporting considerable numbers of chains of tiny coccoid bacterial cells which radiate from the root surfaces. From vetch; slide in soil for 53 days. 1200 ×

FIG. 55. Root hairs radiating from a large rootlet, with large numbers of rod shaped bacteria developing about the root hairs and about the larger root. From mangel beet; slide in soil for 29 days. 650 ×

FIG. 56. Root formation similar to that in figure 55. Colonies of small rod-shaped bacteria as well as scattered cells about the root hairs. From mangel beet; slide in soil for 29 days. 650 ×

FIG. 57. Actinomycete filaments radiating from rootlet (right). The branched filaments bear typical, compact, coiled, sporulating bodies. From tomato; slide in soil for 23 days. 650 ×

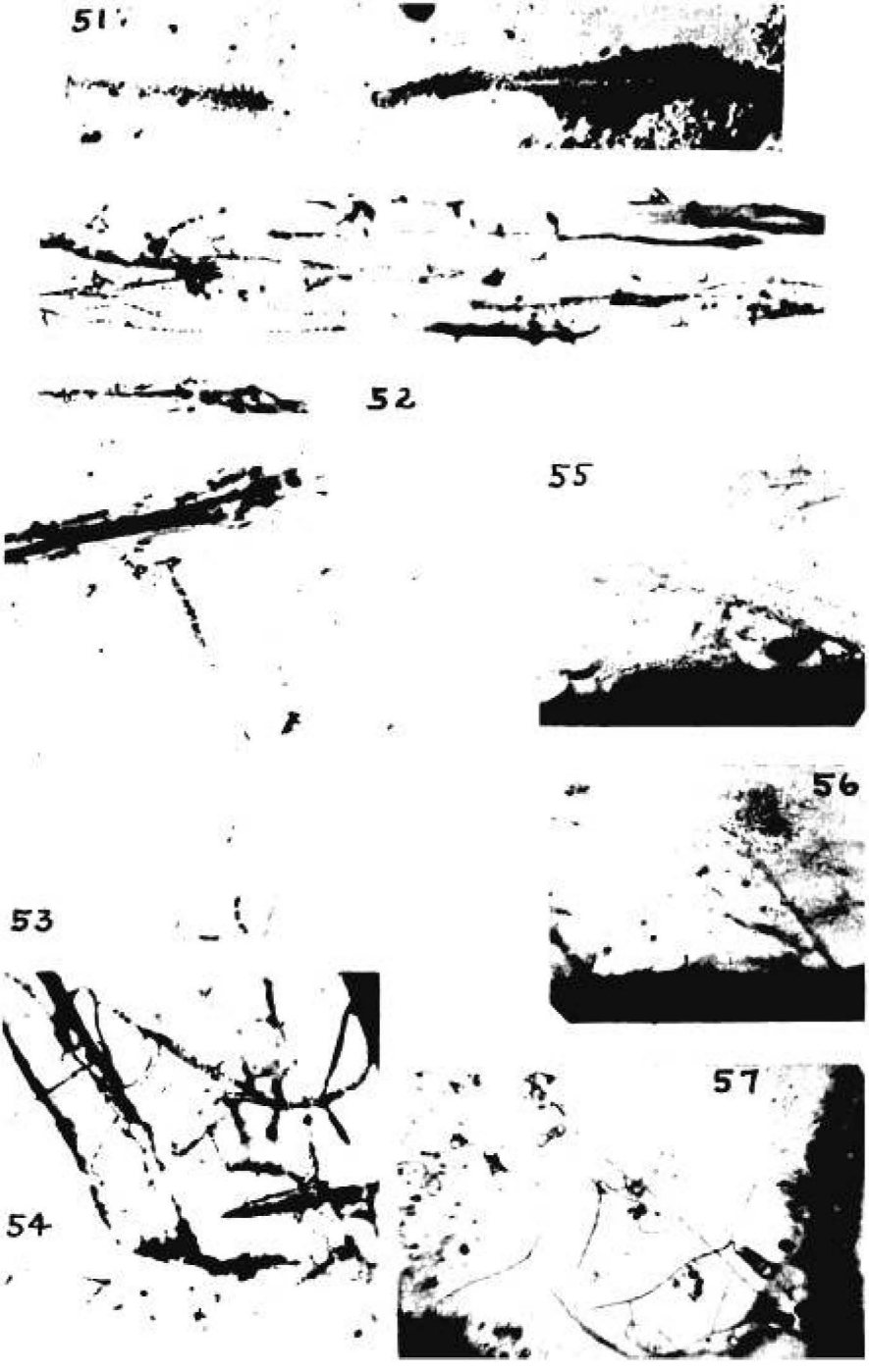
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PLATE 9

FIG. 58. Root hair with considerable numbers of deeply stained rod shaped bacteria in colonies and in short chains along the surface. Small spherical colonies of bacteria also a short distance from the root. From maize; slide in soil for 40 days. 1200 ×

FIG. 59. Colonies of rod shaped bacteria about a clear zone from which a rootlet may have been detached during preparation of the slide. From mangel beet; slide in soil for 29 days. 650 ×

FIG. 60. Root hair with scattered tiny coccoid cells (upper left, on and about the root hair) and radiating, branched, actinomycete filaments. From maize; slide in soil for 40 days. 1200 ×

FIG. 61. Root hairs undergoing extensive attack by bacteria and actinomycetes. Fine branched filaments and larger, more deeply staining rods. Root hairs almost entirely decomposed. From vetch; slide in soil for 71 days. 1200 ×

FIG. 62. Small portion of a large rootlet, which has been almost completely decomposed, with profuse development of chains of small coccoid cells, probably bacteria. From vetch; slide in soil for 53 days. 1200 ×

PLATE 9

PLATE 10

FIG. 63. Root hairs almost entirely decomposed and various scattered microbial cells, including rod shaped bacteria, actinomycete conidia, and fungus spores (left center). From vetch; slide in soil for 71 days. 1200 ×

FIG. 64. Advanced stage of decomposition of root hairs showing abundance of rod shaped bacteria, actinomycete filaments, short pieces of fungus mycelium, and a few fungus spores. From vetch; slide in soil for 71 days. 1200 ×

FIG. 65. Portion of a large root with a dense colony of small coccoid bacterial cells upon it and another loose aggregate a short distance away. From rape; slide in soil for 126 days. 1200 ×


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PLATE 11

FIG. 66. Scattered bacterial cells and actinomycete filaments about a large root. The large projection at the lower right is the head part of a nematode which was upon the root surface. From vetch; slide in soil for 53 days. 1200 ×

FIG. 67. A rootlet covered with great numbers of rod shaped bacteria (or actinomycete conidia?); swellings are formed by the large accumulations of cells. Shadowy outlines of actinomycete filaments can be seen about the rootlet. From vetch; slide in soil for 37 days. 1200 ×

FIG. 68. Rootlet completely covered with rod shaped bacterial cells (actinomycete conidia?). Filaments of an actinomycete extend from the rootlet, one of which bears a spiral coil completely fragmented into conidia. From vetch; slide in soil for 37 days. 1200 ×


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